CONCEPT LIFE SCIENCES

DELIVERING SCIENCE

DATA SHEET

CYTOTOXICITY

The cytotoxic potential of a new chemical entity can be evaluated through the cell culture cytotoxicity assay, which measures viability of the epithelial cell line HepG2 in the presence of a test compound at different concentrations. A number of indicators of healthy function of cells are available: LDH, Neutral red or MTS where signal produced is directly proportional to the number of viable cells.

The assay provides an IG_{50} (concentration that causes inhibition growth of the 50% of the cell population) by means of a 7-point curve. We deliver the IG_{50} concentration of test compound.

CUSTOMER PROVIDES

Compound identifier and molecular formula.

Test: 1-2mg solid or 100uL 10mMDMSO stock.

TEST COMPOUND REPLICATES

n=3 at each concentration (flexible).

FORMAT

96-well microplate, 100µL incubation volume, HepG2 cells are typically used, however other cell lines are available upon request.

PROTOCOL

Day 1: Cells at 80% confluency are diluted to 50,000 cells/mL in EMEM complete media, and a volume is aliquoted into each well (5,000 cells/well). Plate is incubated to allow cells to attach overnight at 37°C and 5% CO_2 humidified atmosphere.

Day 2: Sterile test compound 10mM DMSO stock is used to prepare concentration range 0, 1, 5, 25, 50, 75, & 100µM in EMEM complete media such that the final DMSO concentration is 1% at each concentration.

Media in the plated cells is exchanged for fresh media containing the test compound concentration range (100µL per well). The plate is incubated for the allotted time; 24, 48, 72 hr -depending on client requirements. Compound solutions are refreshed every 24hr to compensate for "edge" effects due to evaporation.

Final Day: The detection solution is prepared. Media from the plated cells is replaced with fresh EMEM complete media. This compensates for volume variation due to evaporation, and minimises the potential for test compound interference during the final detection by plate reader. Detection reagent is then added to each well.

CONTROLS

Digoxin is used as the standard quality control compound in each assay run.

Blank wells are prepared containing the solvent matrix: EMEM complete media and 1% DMSO, but no cells or test compound. These wells are treated with detection reagent exactly like the wells containing the cells.

QUANTITATION

BMG labtech Clariostar plate reader at appropriate wavelength to generate absorbance. Absorbance, is directly proportional to the number of viable cells.

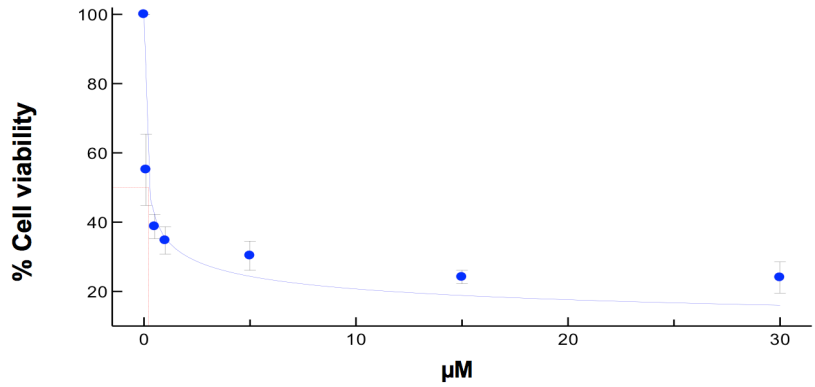
DATA ANALYSIS AND RESULTS

Test compound and Zero absorbances are corrected by subtracting the mean absorbance of the blank wells. The corrected absorbance value of the Zero (0µM test compound concentration) represents 100% viability. All wells containing test compound are compared with this well to calculate viabilities as follows:

$$\text{Viability} = \frac{\text{Corrected Test Absorbance}}{\text{Corrected Zero Absorbance}} \times 100\%$$

The % viability is plotted against test compound concentration, including zero, using curve fitting software, XLfit. The IG_{50} is determined from the graph as the concentration giving 50% viability.

DIGOXIN HEPG2 CYTOTOXICITY IG_{50}



CONCEPT HEPG2 CYTOTOXICITY IG_{50}